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Research Paper

Manufacturing Nanosized Fenofibrate by Salt Assisted Milling

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Purpose. The aim of this study is to develop a new process for manufacturing a nano-sized form of the popular cholesterol-reducing drug fenofibrate which can be implemented on industrial scale with minimal changes of currently used production schemes.
Methods. Salt-assisted milling was used to reduce particle size of commercial fenofibrate from micron-sized particles to nanometer domains.
Results. The optimal parameters for the salt milling are reported, allowing one to reduce the particle size from tens of micrometers to a hundred of nanometers. Dissolution of nano-sized fenofibrate was studied in various formulations and compared against the micron-sized commercially available fenofibrate.
Conclusions. The nano-sized fenofibrate demonstrates faster dissolution kinetics in aqueous media, simulating stomach environment, within the first 60 min as compared to the micronized form. The highest dissolution rate is achieved with the nano-sized fenofibrate when surfactants, such as sodium dodecyl sulfate or inclusion complex forming agents such as alpha-cyclodextrin, are used.

KEY WORDS: dissolution kinetics; fenofibrate; milling; nano-sized drug.

INTRODUCTION

Poor solubility and bioavailability of drugs (1) have been a major concern in the pharmaceutical industry for many years. As much as 90% of therapeutics approved since 1995 have poor water solubility or are lipophilic. Approximately 16% of all drugs have underperformed for these reasons (2). Additionally, the dissolution rate should be maximized for orally or injection-delivered drugs, especially when an immediate response by the patient to the drug is critical.

Solubility and dissolution rate of a drug in physiological environment depend on many factors. Most important are: molecular structure, which determines whether the drug molecules are hydrophilic or hydrophobic; particle surface area (3,4), particle size (5), and diffusion boundary layer thickness h; the last can be influenced by changing the hydrodynamics of the dissolution process.

Reducing drug particle size to micrometer scale and the corresponding increase in the surface area is currently achieved by means of micronization, a process utilizing mechanical comminuting techniques (milling, crushing and grinding). An alternative approach used to micronize drugs is through supercritical fluids that dissolve the drug under elevated pressures and temperatures, resulting in a homogeneous supercritical solution which is ejected through a nozzle, forming drug microparticles (6). While micronization is commonly used to improve solubility, there are many drugs which require further decrease in particle size down to the nanometer range in order to reach the required dissolution rate. Nanocrystals of some drugs can be produced by precipitation (7). In this approach, the drug is dissolved in a solvent which is subsequently added to a nonsolvent in order to precipitate the crystals. However, this technique is difficult to control, as crystal growth needs to be stopped before the formation of microcrystals. Crystal growth inhibitors, which are used for this purpose, bring in the subsequent problem of purification. In addition, this technique requires changing the established drug manufacturing procedures, which may not always be possible. Due to these considerations, more desirable approach would be to keep the current drug synthesis schemes unchanged and employ scaling down techniques capable of reducing the particle size of the synthesized drugs from millimeters or micrometers to nanometers, without contaminating the drug with difficult-to-remove organic or inorganic impurities.

A typical example of a poorly soluble drug is a popular cholesterol-lowering drug fenofibrate (Fig. 1a) which limits the availability of fatty acids for triglyceride synthesis, stimulates reverse cholesterol transport and lipoprotein lipase activity, and suppresses the activity of HMG-CoA reductase in the liver (8). Fenofibrate is administered orally and is hardly soluble in aqueous media (solubility in deionized (DI) water is less than 0.0008 g/L (9)). As a consequence, the absolute bioavailability of fenofibrate cannot be determined. Previous data by Munoz *et al.* (10) showed enhanced absorption of the micronized form of fenofibrate as compared to the non-micronized formulation.

In this study we demonstrate an increase in the dissolution rate of fenofibrate *in vitro* by reducing its particle

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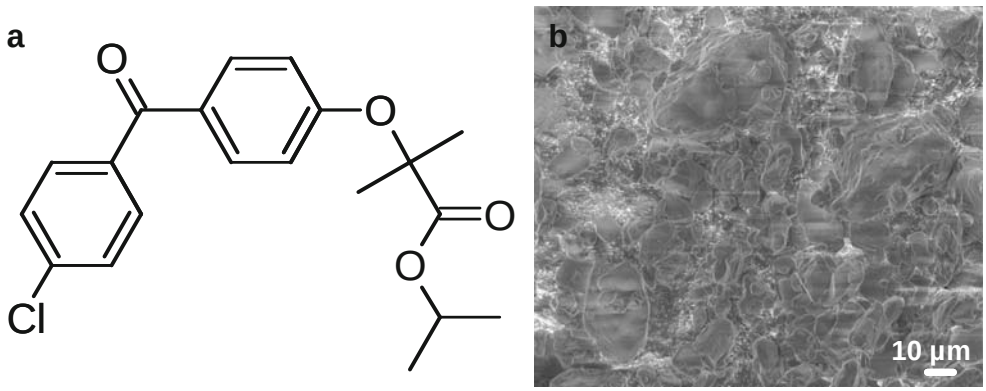

Fig. 1. Chemical structure (**a**) and SEM image of as-received fenofibrate (**b**) showing a large crystal size and a broad particle size distribution.

size to the nanometer range using salt-assisted milling. We are not aware of any prior published reports on using salt-assisted milling to downsize fenofibrate or any other drugs. The idea to use salt as a milling body instead of zirconia or other ceramic microbeads, which are commonly used today, has several advantages. First, NaCl is hard enough to crush soft organic materials, including fenofibrate and other drugs. Second, NaCl is milled by steel balls during the drug milling process, thus the size of NaCl crystals decreases continuously along with the drug from micrometers down to nanometer range, significantly improving the efficiency of drug milling and preventing its re-agglomeration. Third, NaCl can be easily washed out with water to negligible levels after milling, in contrast to zirconia or silica, which are insoluble and contaminate the product. Finally, NaCl is inexpensive, non-toxic and absolutely safe.

MATERIALS AND METHODS

Materials

Fenofibrate (>99% pure) and sodium chloride (99.5% pure, particle size ~300 μm) were purchased from Sigma Aldrich, USA and used without subsequent purification.

Methods

A temperature controlled lab attrition mill with stainless steel grinding media of sizes, 0.478 cm (1/6 in.) and 0.635 cm (1/4 in.) was designed and manufactured for this project by Union Process, Inc., USA, and used for milling (Fig. 2a). A typical procedure included mixing the commercial fenofibrate composed of particles of irregular shape and size ranging within 20–500 μm (Fig. 1b) with NaCl and subsequent milling of the mixture. NaCl acts as an additional milling medium, which is harder than fenofibrate, but which itself is still milled by steel balls. Thus, its particle size decreases simultaneously with that of fenofibrate, allowing a finer milling of the drug. In addition, NaCl separates fenofibrate particles preventing their reagglomeration.

Dissolution tests were performed using a custom-design dissolution apparatus (Fig. 2b) in 0.1 M aqueous HCl at 37°C to simulate stomach conditions. The kinetics of fenofibrate dissolution was monitored by absorption at 290 nm using Lambda 40 UV–Vis Spectrometer (PerkinElmer, USA).

The BET specific surface area (SSA) measurements on the samples were done with nitrogen at 77 K using a Quadrasorb surface area and pore size analyzer manufactured by Quantachrome, USA.

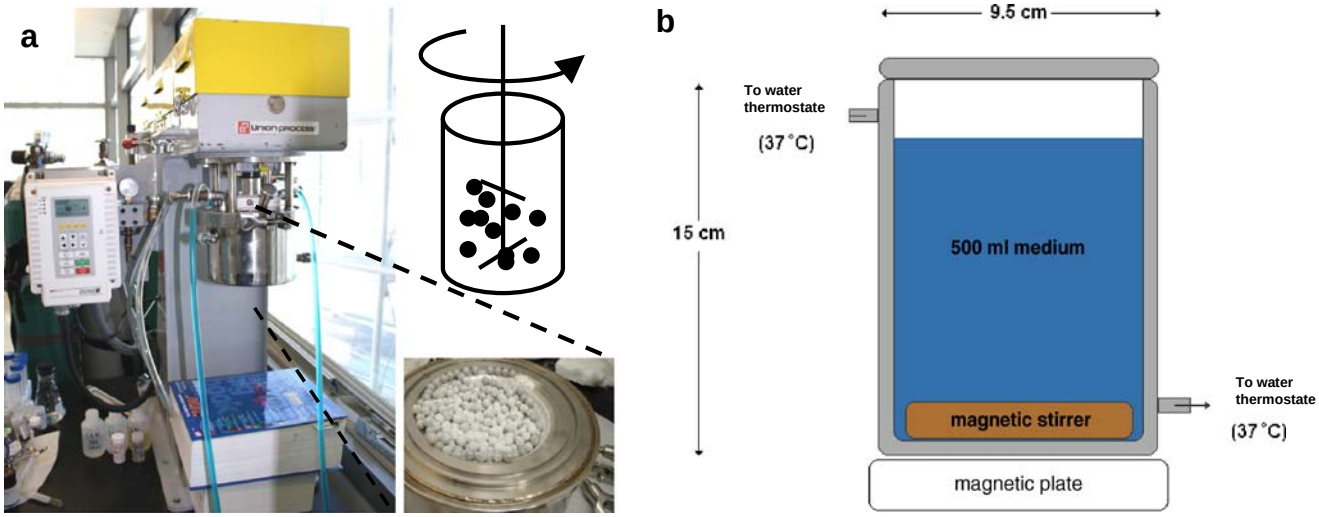

Fig. 2. Attritor mill (**a**) and dissolution apparatus (**b**) used in this study.

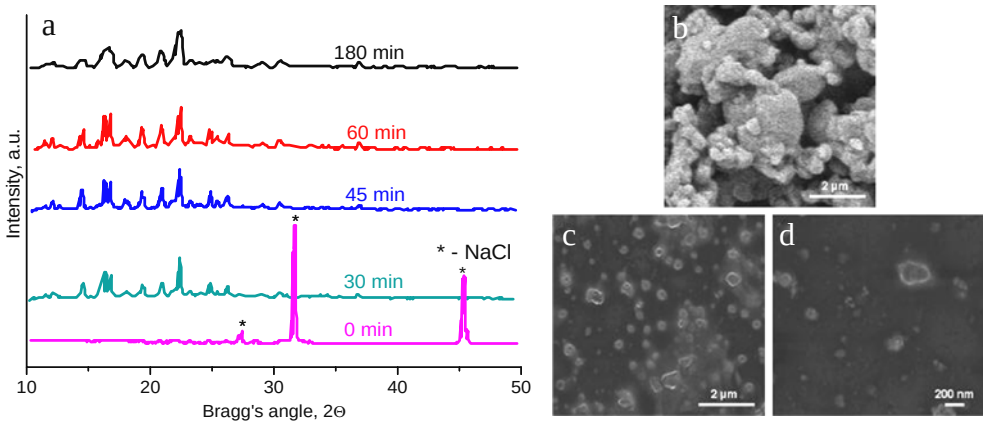

Fig. 3. XRD patterns (**a**) and SEM images of the milled product after milling at 500 rpm for 45 min (**b**), 60 min (**c**), and 180 min (**d**) and NaCl dissolution. 0 min corresponds to a mixture of fenofibrate and NaCl before milling.

Particle size before and after milling was estimated by scanning electron microscopy (SEM) using a Zeiss Supra 50VP SEM in a variable pressure mode under low accelerating voltage. In addition, the particle size has been calculated from the surface area measurements assuming a spherical shape for the particles.

X-ray diffraction (XRD) analysis was done using a Siemens D500 X-ray powder diffractometer with a 1,500 W Cu fine focus tube operated at 30 mA and 40 kV. The sample for XRD was adhered gently on a pre-frosted glass slide (Fischer Scientific). No additives were used for sample mounting.

RESULTS

Parametric studies of the milling conditions on the fenofibrate particle sizes were conducted. It should be mentioned that without salt added, fenofibrate can not be milled with the crushing balls of the sizes we use. Four parameters: milling time, milling speed, drug-to-salt ratio, and crushing ball size were each subjected to variation, keeping the remaining parameters constant. Subsequently the particle size and dissolution profiles of the milled fenofibrate were analyzed.

Dependence on Time of Milling

The commercial sample of fenofibrate was milled with sodium chloride in a 110 cm^3 attrition mill jar at 500 rpm, for 30 min, 45 min, 60 min and 180 min. For the milling series, 0.635 cm balls were used and the mill chamber temperature was maintained at 0°C to avoid heating and degradation of the drug. The drug-to-salt ratio was maintained at 1:7 for the entire series. Once milled, the extract was repeatedly rinsed with DI water and centrifuged till there were no traces of NaCl in the water after rinsing, according to a standard visual test for Cl^- with $AgNO_3$. XRD patterns and SEM images of the milled and washed products from this series are shown in Fig. 3. While the smallest particles could be lost during purification, the general trend of particle size change *vs.* milling time was very pronounced. As the milling time increased, there was a reduction in particle size, observed with SEM and manifested in a broadening of XRD peaks. While in general the broadening of XRD peaks may result from both decrease in crystal size and amorphization of a material, in our case it is rather a result of the smaller size as revealed by SEM and by the absence of hallo at low diffraction angles in XRD typical for amorphous materials. According to SEM, 30 min milling yielded particles as small as 800 nm (not shown), 45 min sample showed 500 nm particles, and 60 min of milling resulted in particles smaller than 500 nm. The next sample was milled for 3 h, yielding the smallest particles of less than 100 nm. Table I shows corresponding values of SSA and calculated average particle size. It is important to mention that the results derived from SSA are statistically more reliable compared to SEM, because of a much larger volume of material tested, but those may be affected by agglomeration of nanoparticles, which hinders adsorbate gas access to the surface of primary particles in the agglomerates, and by deviations of the shape of real particles from the assumed spherical shape. As expected, the decrease

Table I. BET SSA, Particle Size, and Melting Point of the As-received and Milled Fenofibrate

Milling conditions	BET SSA, m^2/g	Particle size (calculated from SSA)	Melting point, °C
As-received	0.074	63 μm	83.26
Milling time, min			
30	0.1	47 μm	81.42
45	4.0	1.2 μm	80.49
60	6.8	700 nm	80.46
180	29.0	165 nm	80.44
Milling speed, rpm			
500	6.8	700 nm	80.46
600	1.8	2.6 μm	80.49
700	0.2	23 μm	81.67
Drug-to-salt ratio			
1:2	3.0	1.61 μm	80.56
1:7	6.8	700 nm	80.46
1:12	16.13	300 nm	80.46
Crushing balls diameter, cm			
0.635	6.8	700 nm	80.46
0.478	9.0	530 nm	80.44

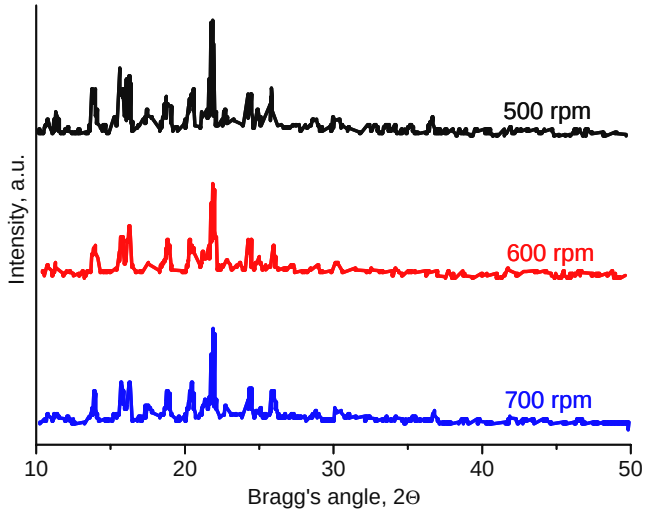

Fig. 4. XRD patterns of the products milled for 1 h at 500 rpm, 600 rpm, and 700 rpm.

in particle size during milling is accompanied by a slight lowering in the fenofibrate melting point (Table I).

Dependence on Speed of Rotation

Commercial fenofibrate was milled with sodium chloride in a 110 cm^3 attrition mill at varying speeds of rotation. For the milling series, 0.635 cm balls were used for 1 h and the temperature was maintained at 0°C. The drug-to-salt ratio was maintained at 1:7 for the entire series. The milling experiments were done at three speeds: 500, 600, and 700 rpm. After the milling, the samples were washed and the absence of NaCl was verified as described above. The SEM images and XRD patterns of the milled samples are shown in Figs. 3c and 4, respectively.

As the rotation speed increases, the efficiency of the reduction in particle size decreases. The particles of products milled at higher speed are larger than at lower speeds. According to SEM, 500 rpm milling yielded particles as small as 400 nm (Fig. 3c) while the 600 rpm and 700 rpm samples have particles of sizes ~2 μm and ~10 μm respectively (SEM not shown). Table I shows corresponding values of SSA and calculated average particle size. The trend is the same as observed by SEM, although the measured values are somewhat larger. The trend in the particle size change with the rotation speed is accompanied by a corresponding trend in the fenofibrate melting point, confirming that the melting point correlates well with the grain size of fenofibrate.

It is generally accepted that milling speed increases the efficiency of milling (11). However, we observed that the higher speed does not necessarily translate into reduction of particle size. This may be explained by the fact that the lowest speed used, i.e. 500 rpm, could already be at or above the limit for the size reduction, whereas at higher speeds the balls move together in the whipping motion and impede efficient milling. Hence the particle size reduction is less efficient.

Dependence on Drug-to-Salt Ratio

Commercial fenofibrate was milled along with sodium chloride in a 110 cm^3 attrition mill at three drug-to-salt ratios: 1:2, 1:7 and 1:12. For the milling series, 0.635 cm balls were used for 1 h and the temperature was maintained at 0°C. The milling speed was 500 rpm for the entire series. Significant peak broadening in XRD (Fig. 5a), direct observation of particle size with SEM (Figs. 5b, c and 3c), and the data in Table I demonstrate that the particles were milled down to a smaller size when the salt content was increased. However, there is a practical limit to which the fenofibrate can be diluted with salt, because it affects the yield and, subsequently, the cost of the product.

Dependence on the Milling Media Size

Steel balls of two sizes were used for this series: 0.635 cm and 0.478 cm. The drug-to-salt ratio was kept at 1:7 and the milling was done for 1 h at 500 rpm and 0°C. The milled product was washed and tested as described above.

The XRD and SEM results for milled samples are presented in Figs. 6 and 3c. The SEM images demonstrate that the fenofibrate particles are milled down to 700 nm with

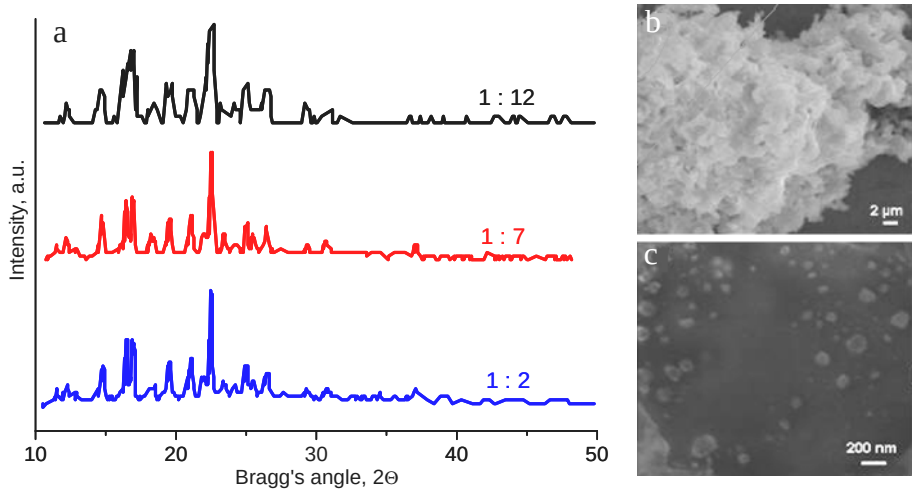

Fig. 5. XRD patterns (**a**) and SEM images of the fenofibrate milled at 1:2 (**b**), 1:7 (for SEM see Fig. 3c), and 1:12 (**c**) drug-to-salt ratio.

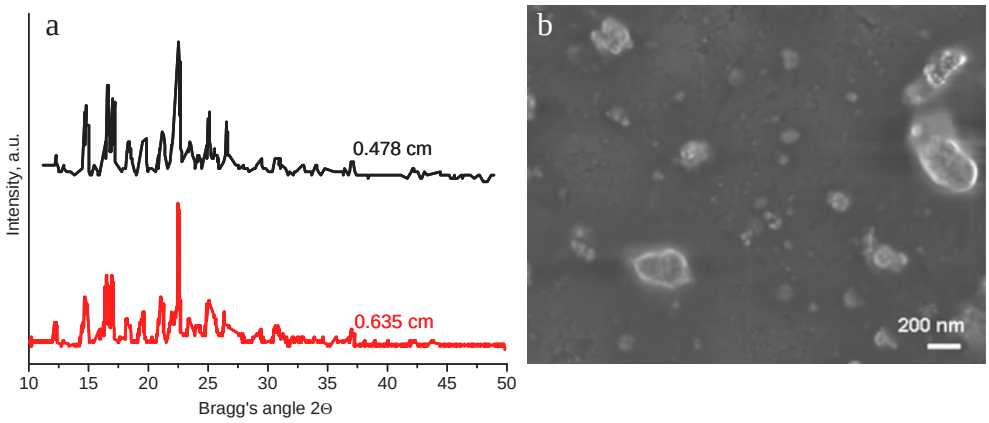

Fig. 6. XRD patterns (**a**) and an SEM image of fenofibrate milled with steel balls of 0.635 cm (for SEM see Fig. 3c) and 0.478 cm (**b**) in diameter.

0.635 cm balls (Fig. 3c) and 300 nm with 0.478 cm balls (Fig. 6b). This supports the conclusion that a smaller particle size can be achieved by using smaller milling media. These results are confirmed by the SSA data in Table I. The BET analysis showed relatively high specific surface area, which corresponds to particle sizes of 700 nm for the 0.635 cm balls' milling experiment and 530 nm for the milling with smaller balls.

Dissolution Tests

Dissolution tests were performed with the apparatus shown in Fig. 2. The concentration of dissolved drug was determined by UV/Vis spectrometry and monitored as a function of time (Fig. 7). It should be noted that due to a limit in time during which the drug can be resided in a human stomach, only the initial dissolution rate and concentration was of interest from a practical point of view. Therefore, we restrained the dissolution rate analysis only by data measured for the first 1 h.

Within this period of time the dissolution rates are slightly higher for the milled samples (curves 5 and 6) in comparison to the commercial sample (curve 7), with finer particles enabling faster dissolution. Unwashed milled fenofibrate with high content of sodium chloride (curve 6) demonstrates lower dissolution rate and concentration achieved within 1 hour due to a salting-out effect of NaCl. However, the overall differences between the commercial (curve 7), washed salt-milled (curve 5), and unwashed salt-milled fenofibrate (curve 6) are subtle and far from being considered practically important. In the next section we discuss the ways to reveal the advantages of nanoscale fenofibrate particles and achieve the expected improvement in dissolution rate.

DISCUSSION

Fenofibrate is a highly hydrophobic substance and therefore, in hydrophilic media the nanometer-sized fenofibrate particles tend to assemble themselves in micron-sized and larger aggregates held together by hydrophobic interactions. Thus, the aggregation cancels all the advantages in dissolution behavior one expects for nanometer sized particles as compared to micrometer sized ones. In this situation surfactants were proved to be effective to prevent the aggregation of nanoparticles and to achieve the desired effect (12). Indeed, the curves 1–4 in Fig. 7 show that small additions of common surfactants such as sodium dodecyl sulfate (SDS) or inclusion complex forming agents such as α-cylcodextrin result in a significant increase in the dissolution rate of all fenofibrate samples. The increase in concentration, achieved within the first 60 min for the washed salt-milled nanometer-sized fenofibrate compared to the commercial micrometer-sized sample is from 5 to $6 \cdot 10^{-4}$ mg/mL when SDS is used (curves 3 and 1 in Fig. 7), and from 3 to $6 \cdot 10^{-4}$ mg/mL when α-cylcodextrin is used (curves 4 and 2 in Fig. 7). Thus, the use of surfactant and drug–cyclodextrin inclusions helps in preventing the agglomeration of the nanosized drug particles and fully reveals the effect of nanoscale particle size on dissolution rate of fenofibrate. The effect of solubilizing agents is found to be sensitive to the type of the agent: SDS works well for both the commercial and salt-milled fenofibrate whereas the α-cylcodextrin works much better for the salt-milled form of drug. However, the maximal dissolution rate in both cases is achieved only for salt-milled nanometer-sized

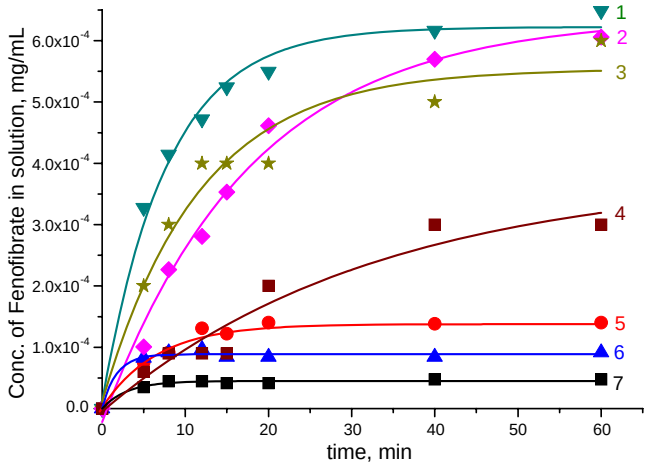

Fig. 7. Dissolution profiles of fenofibrate milled with NaCl for 1 h, 500 rpm, 0.25-in. ball size in comparison with commercial powder: *1*—salt-milled in 0.025 M SDS; *2*—salt-milled in 0.01 M α-cyclodextrin; *3*—commercial in 0.025 M SDS; *4*—commercial in 0.01 M α-cyclodextrin; *5*—salt-milled; *6*—salt-milled unwashed; *7*—commercial.

form of fenofibrate. In this case the use of either SDS or α-cylcodextrin reveals the advantage of nanoscale fenofibrate particles and brings the concentration of fenofibrate close to saturation limit faster than in case of the micron-sized commercial sample.

CONCLUSIONS

Salt-assisted attrition milling with steel balls was demonstrated as a potential technique to produce a nanosized form of fenofibrate. A parametric study of milling process has shown that the optimal conditions to produce fenofibrate nanoparticles are: milling time 60–180 min, speed of rotation 500 rpm, drug-to-salt ratio 1:7–1:12, and crushing media size 0.478–0.635 cm. Dissolution profiles of milled particles studied in a 0.1 N HCl solution simulating stomach conditions show only a slight increase in the dissolution rate within the first hour with decreasing particle size. This is explained due to aggregation of hydrophobic fenofibrate particles dispersed in aqueous electrolyte solution. Small additions of commonly used surfactants such as sodium dodecyl sulfate or inclusion complex forming agents such as cyclodextrins were shown to reveal the crucial role of nanoscale particle size in increasing the dissolution rate of the salt-milled fenofibrate as compared to the commercial drug.

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REFERENCES

1. G. L. Amidon, H. Lennernas, V. P. Shah *et al.* A theoretical basis for a biopharmaceutic drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. *Pharm. Res*. **12**(3):413–420 (1995) doi:10.1023/A:1016212804288.
2. R. D. Connors, and E. J. Elder. Using a portfolio of particle growth technologies to enable delivery of drugs with poor water solubility. *Drug Deliv. Technol*. **4**(8):78–84 (2004).
3. E. Merisko-Liversidge, G. G. Liversidge, and E. R. Cooper. Nanosizing: a formulation approach for poorly-water-soluble compounds. *Eur. J. Pharm. Sci*. **18**(2):113–120 (2003) doi:10.1016/S0928-0987(02)00251-8.
4. A. P. Tinke, K. Vanhoutte, R. De Maesschalck *et al.* A new approach in the prediction of the dissolution behavior of suspended particles by means of their particle size distribution. *J. Pharm. Biomed. Anal*. **39**(5):900–907 (2005) doi:10.1016/j.jpba.2005.05.014.
5. H. M. Deng, J. Ding, Y. Shi *et al.* Ultrafine zinc oxide prepared by precipitation/mechanical milling. *J.Mater. Sci*. **36**(13):3273–3276 (2001) doi:10.1023/A:1017902923289.
6. E. Reverchon, and G. Della Porta. Supercritical fluids-assisted micronization techniques. Low-impact routes for particle production. *Pure Appl. Chem*. **73**(8):1293–1297 (2001) doi:10.1351/pac200173081293.
7. H. Sucker, and P. Gassmann. Improvements in pharmaceutical compositions. GB Patent 2269536A, 1994
8. J. Shepherd. Mechanism of action of fibrates. *Postgrad. Med. J*. **69**(Suppl. 1):S34–S41 (1993) doi:10.1136/pgmj.69.807.80.
9. S. Jamzad, and R. Fassihi. Role of surfactant and pH on dissolution properties of fenofibrate and glipizide—a technical note. *AAPS Pharm. Sci. Tech*. **7**(2):Article 33 (2006) doi:10.1208/pt070233.
10. A. Munoz, J. P. Guichard, and P. Reginault. Micronised fenofibrate. *Atherosclerosis*. 110(Suppl):S45–S48 (1994) doi:10.1016/0021-9150(94)05375-S.
11. C. Mochales, H. El Briak-BenAbdeslam, M. P. Ginebra *et al.* Dry mechanochemical synthesis of hydroxyapatites from DCPD and CaO: influence of instrumental parameters on the reaction kinetics. *Biomaterials*. **25**(7–8):1151–1158 (2003) doi:10.1016/j.biomaterials.2003.08.002.
12. C. Noory, N. Tran, L. Ouderkirk *et al.* Steps for development of a dissolution test for sparingly water-soluble drug products. *Am. Pharm. Rev*. **5**(4):16–21 (2002).